Microtubule stabilising agents and ionising radiation: Multiple exploitable mechanisms for combined treatment

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Abstract: Combined radiochemotherapy treatment modalities are in use for many indications and there-fore of high interest. Even though a combined modality in clinical use is often driven by prag-matic aspects, mechanistic preclinical-based concepts of interaction are of importance in order to translate and implement an optimal combination and scheduling of two modalities into the clinics. The use of microtubule stabilizing agents is a promising strategy for anti-cancer therapy as a part of combined treatment modality with ionizing radiation. Traditionally, microtubule targeting agents are classified as cytotoxic chemotherapeutics and are mostly used in a maxi-mally tolerated dose regimen. Apart from direct cytotoxicity and similar to mechanisms of mo-lecular targeting agents, microtubule stabilizing agents interfere with multiple cellular process-es, which can be exploited as part of combined treatment modalities. Recent preclinical investi-gations on the combination of ionizing radiation and microtubule stabilizing agents reveal new mechanistic interactions on the cellular and tumor level and elucidate the supra-additive tumor response observed particularly in vivo. The major focus on the mechanism of interaction was primarily based on radiosensitization due to cell cycle arrest in the most radiosensitive G2/M-phase of the cell cycle. However, other mechanisms of interaction such as reoxygenation and direct as well as indirect endothelial damage have also been identified. In this review we sum-marize and allocate additive and synergistic effects induced by the combined treatment of clini-cally relevant microtubule stabilizing agents and ionizing radiation along a described radiobio-logical framework encompassing distinct mechanisms relevant for exploiting the combination of drugs and ionizing radiation.

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Microtubule Stabilizing Agents and Ionizing Radiation: Multiple Exploitable Mechanisms for Combined Treatment

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Abstract

Combined radiochemotherapy treatment modalities are in use for many indications and therefore of high interest. Even though a combined modality in clinical use is often driven by pragmatic aspects, mechanistic preclinical-based concepts of interaction are of importance in order to translate and implement an optimal combination and scheduling of two modalities into the clinics. The use of microtubule stabilizing agents is a promising strategy for anti-cancer therapy as a part of combined treatment modality with ionizing radiation. Traditionally, microtubule targeting agents are classified as cytotoxic chemotherapeutics and are mostly used in a maximally tolerated dose regimen. Apart from direct cytotoxicity and similar to mechanisms of molecular targeting agents, microtubule stabilizing agents interfere with multiple cellular processes, which can be exploited as part of combined treatment modalities. Recent preclinical investigations on the combination of ionizing radiation and microtubule stabilizing agents reveal new mechanistic interactions on the cellular and tumor level and elucidate the supra-additive tumor response observed particularly *in vivo*. The major focus on the mechanism of interaction was primarily based on radiosensitization due to cell cycle arrest in the most radiosensitive G2/M-phase of the cell cycle. However, other mechanisms of interaction such as reoxygenation and direct as well as indirect endothelial damage have also been identified. In this review we summarize and allocate additive and synergistic effects induced by the combined treatment of clinically relevant microtubule stabilizing agents and ionizing radiation along a described radiobiological framework encompassing distinct mechanisms relevant for exploiting the combination of drugs and ionizing radiation.

Keywords: Ionizing Radiation, Microtubule Stabilizing Agents, Radiochemotherapy, Epothilones, Taxanes

Introduction: Microtubule Stabilizing Agents

Microtubule targeting agents belong to the most important classes of anti-cancer agents and are subdivided in two groups, according to their mode of action. While microtubule destabilizers prevent the assembly of tubulin heterodimers, microtubule stabilizing agents (MSA) prevent the shortening of microtubules resulting in the accumulation of polymerized microtubule bundles and the interference of the mitotic spindle function.(1-4) Experimental evidence concerning the kinetics and mechanism of tubulin-binding as well as the ability to actively promote microtubule function by paclitaxel mimetics has been recently provided using biochemical and NMR techniques.(5) Eventually both classes of microtubule targeting agents alter spindle-microtubule dynamics, which results in a transient or permanent M-phase arrest and the induction of apoptotic cell death or mitotic catastrophe (Figure 1).(6) In this review we will specifically focus on the mode of interaction between MSA and ionizing radiation as part of a combined treatment modality.

Taxanes and epothilones are the clinically most relevant microtubule stabilizing agents. The *taxanes* (paclitaxel and docetaxel) have been approved for a broad range of indications, including advanced breast cancer after failure of combination chemotherapy or at early relapse,(7) high grade ovarian cancer in combination with platinum compounds, and primary treatment of non-small cell lung cancer in combination with cisplatin.(8) Furthermore paclitaxel is used in an "off-label manner" for other tumor types, such as cancer of unknown origin, bladder, esophagus, gastric, head and neck, and cervical cancers (reviewed in (9)). Paclitaxel has also been evaluated clinically for its radiosensitizing properties for various tumors(10-14) and drug plasma concentrations in patients. Low concentrations with prolonged exposure during long parts of the course of radiation therapy have been found feasible and tolerated in patients.(12-18) Docetaxel is used as first-line chemotherapy for locally advanced or metastatic breast cancer,(19) nonresectable, advanced or metastatic non-small cell lung cancer after failure of cisplatin-based therapy, hormone-refractory metastatic prostate cancer in combination with prednisone,(20) gastric adenocarcinoma in combination with cisplatin and 5-fluorouracil,(21) and inoperable

advanced squamous cell cancer of the head and neck in combination with cisplatin and 5-fluorouracil. Both of the approved taxane derivates are hydrophobic and require organic solvents for administration (cremophor EL / ethanol, polysorbate / ethanol), which by themselves can cause unwanted side effects.(22) The *epothilones* are nontaxoid macrolide MSA of bacterial origin, which share the same binding site on beta-tubulin (in close proximity to residue Thr274) with taxanes,(23) albeit with different affinities.(1, 24-26) Clinically different epothilone derivatives are currently in various stages of development as antitumor compounds. Several properties like increased water solubility, low susceptibility to common mechanisms of resistance and the more tolerable toxicity profile, favor their development. Ixabepilone (Ixempra®) is the first approved compound in this class and indicated as monotherapy or in combination with capecitabine for the treatment of patients with metastatic breast cancer. Apart from a manageable safety profile, ixabepilone demonstrates activity after failure and resistance towards anthracycline and taxane standard therapy.(27) Epothilone B (patupilone) was tested as a phase III monotherapy agent against ovarian cancer and other epothilones are undergoing a wide spectrum of single and combined treatment modality in phase II studies (e.g. for recurrent glioblastoma, CNS metastases from breast cancer, prostate, cervical, renal cell, gastric and lung tumor, as well as non-Hodgkin's Lymphoma (www.cancer.gov).(15, 28-31)

Both, taxanes and epothilones, have been extensively tested at the preclinical level in combination with ionizing radiation, demonstrating a strong supra-additive treatment response. Here, we will outline classic rationales for the combined treatment modality of ionizing radiation with microtubule stabilizing agents and discuss novel mechanistic preclinical-based concepts of interaction between these two modalities.

Rationale for the Combined Use of Irradiation and Cytotoxic Agents

The rationale for a combined treatment with ionizing radiation (IR) and chemotherapy is to increase survival by improving locoregional tumor control and decrease the probability of distant

disease, with concurrent organ and function preservation.(32, 33) Recently, a new framework encompassing five distinct mechanisms relevant for exploiting the combination of drugs and ionizing radiation has been proposed.(34) While for some of the mechanisms direct drug-radiation interactions at the tumor-cellular level is not required (e.g. *in spatial cooperation, normal tissue protection*), other mechanisms are fundamentally based on their mutual interaction (e.g. *cytotoxic enhancement, biological cooperation, temporal modulation*). With regard to microtubule stabilizing agents, IR-MSA interactions can be allocated to several of these mechanisms. Interestingly these mechanisms even complement each other on several levels thereby further enhancing the potency of this promising combined treatment modality.

The main rationale to exploit *spatial cooperation*, combining a drug with efficacy against systemic disease with radiotherapy against locoregional disease, is to achieve local *and* systemic control by full doses of both treatment modalities, often applied in sequence.(20) For the mechanisms involving a direct drug-radiation interaction at the cellular level (*cytotoxic enhancement, biological cooperation and temporal modulation*), the strategies aim mainly at enhancing cell killing, interfering with repair mechanisms, targeting distinct cell populations (e.g. hypoxic cells) and microenvironmental structures within the tumor.

Interactions between Ionizing Radiation and MSA (Figure 2)

Cell cycle specific enhancement (cytotoxic enhancement). MSAs induce mitotic arrest in most tumor cells. Depending on the dose applied and/or the genetic background and inherent cellular sensitivity of the tumor cells, MSA-induced mitotic arrest will lead to transient cell cycle arrest or apoptosis. Therefore only a poor correlation exists between MSA-induced mitotic arrest and tumor control, as mitotically arrested cells in some tumors are capable of continued survival (35, 36). In paclitaxel- and docetaxel-resistant tumors, the radioenhancing mechanism is primarily based on the transient accumulation of cells in this cell cycle phase.(36, 37)

The cell cycle phases of late G2 and mitosis are most sensitive to ionizing radiation.(38, 39) Ionizing radiation produces different types and quantities of chromosomal aberrations at various stages of the cell cycle. The frequency of IR-induced chromosomal aberrations is higher for cells in G2- and M-phase than for cells irradiated in the G1- and S-phase of the cell cycle.(40) In addition, cells in late G2/M-phase already passed the G2-checkpoint to repair their DNA damage, and thus the frequency of residual, detrimental chromosomal aberrations in cells entering mitosis is also increased. As mitotic cells with double strand breaks will lose genetic material following cell division, irradiation of MSA-treated, transiently arrested late G2-/M-phase-cells will result in genomic instability and eventually mitotic catastrophe.(35, 41-45)

This implies that the therapeutic efficacy of the combined treatment modality strongly depends on the innate cellular drug sensitivity and in particular on the combination scheduling. Indeed, effects ranging from supra-additive interactions (Figure 3), to additive, and even sub-additive effects are documented.(43, 46-50) The radiosensitizing effect of MSA is most prominent between 8-12 hours after start of drug exposure at the time of accumulation of cells in G2/M-phase (Figure 4).(42, 43, 49, 50) In contrary, the cytotoxic M-phase-related potency of paclitaxel added *after* irradiation was lost and did not increase IR-induced cell death due to the strong IR-induced G1- and G2- cell cycle arrest and subsequent sublethal damage repair.(49, 51) *In vivo*, multiple doses of taxanes given during the course of fractionated irradiation maximized the exposure for cells to ionizing radiation even in drug resistant tumors.(52) However, with intermittent multiple high doses of taxanes, the therapeutic gain was in part limited by side effects in acutely responding tissues such as jejunal mucosa, skin and connective tissues as observed in murine tumor models.(52-54)

Moderate concentrations of MSA, which are sufficient to induce a G2-M cell cycle arrest are optimal for radioenhancement.(43, 47-50) Interestingly, low dose MSA-treatment may also induce an additional G2/M-phase independent mode of radiosensitization. Low doses of patupilone induced a transient accumulation of cells in S phase, but only on combined treatment with ionizing radiation.(55) Combined treatment did not result in the accumulation of cells in the

radiosensitive G2/M-phase and radiosensitization was rather due to an S-phase-related process. A similar effect has also been described for synchronized HeLa cells, in which docetaxel resulted in a S-phase specific (and hence radioresistant) cell killing.(56, 57)

Furthermore there seems to be a tendency toward a concentration-dependent increase in DNA double strand breaks after combined treatment, suggesting a reduction of DSB repair capacity as an additional mechanism of sensitization.(46)

Reoxygenation theory (biological cooperation, temporal modulation). The enhanced radiation response of combined treatment with microtubule stabilizing agents and ionizing radiation has also been attributed to "tumor cell reoxygenation". Massive cell loss due to MSA-induced apoptosis or necrosis might lead to a shortening of the tumor cell-vascular distance and a decrease of the intratumoral fluid pressure (IFP) with a subsequently increased blood delivery to hypoxic tumor regions as well as a decreased overall oxygen consumption rate. Eventually this results in increased tumor oxygenation, and subsequently increased radiation sensitivity (Figure 4).(35, 37, 52, 54, 58, 59) For example, paclitaxel in combination with ionizing radiation significantly reduced tumor hypoxia in MCA-4 xenograft tumors, especially in a drug-radiation interval of 3 days.(59) Due to the high concentration of the administered drug, this effect was mostly attributed to extensive cytotoxicity caused by paclitaxel.

Endothelial theory (biological cooperation, temporal modulation). As the radiosensitivity of the tumor vascular network codetermines the tumor response to IR, enhanced tumor radiosensitivity can be attributed to the effect of microtubule agents on the tumor endothelial system.(60) Microtubule stabilizing agents have anti-angiogenic properties at high and at low, non-tumor cytotoxic doses.(61) These properties are on one hand based on *direct* anti-endothelial cell activity, but also on the *indirect* tumor-cell mediated anti-angiogenic effect, by inhibition and subsequent down-regulation of proteins and genes involved in angiogenesis and hypoxic adaptation.(62, 63)

High doses of microtubule stabilizing agents act in a cytotoxic manner via mitotic block and activation of the mitochondrial apoptotic pathway, while on the other hand, low doses decelerate overall cell cycle progression and inhibit migration and proliferation,(64, 65) which results in reduced capillary-like tube formation.(61, 66) Similar to the radiosensitizing effect on the tumor cell level, prolonged metaphase-to-anaphase transition in endothelial cells can also provoke an increase of the radiation response on the tumor vasculature level by temporal modulation in the classic radiobiological framework of cell cycle redistribution (see above).(34) In addition to the anti-angiogenic effects, MSA such as epothilone B also exert vascular disruptive effects. Drug administration led to a rapid destruction of blood vessels (observable at day two) associated with a decrease in tumor IFP.(67) This direct destruction of blood vessels and decrease of IFP might also lead to a subsequent reorganization of the vasculature, thereby temporarily decreasing the hostility of tumor microenvironment by also changing oxygenation and pH-status of the tumor.(67, 68) Enhanced tumor oxygenation could also be attributed to the proposed concept of tumor vasculature normalization, caused by a transient decrease of VEGF signaling and thereby reduction of vessel abnormalities such as tortuosity and leakage of the impaired tumor vasculature. Addition of radiation therapy during this "window of normalization" would in principle enhance radiation response.(68, 69)

However, reduction of tumor hypoxia on MSA-treatment is not a prerequisite for a supra-additive anti-tumor effect when combined with ionizing radiation. Early results indicated that paclitaxel suppresses the expression of VEGF in murine breast cancer cells *in vivo* and *in vitro.*(70) Our own experiments performed with patupilone in a genetically defined MSA-sensitive and MSA-resistant tumor model revealed that the anti-angiogenic and radiosensitizing effect on the level of the tumor vasculature of MSA is strongly promoted in an indirect tumor-cell mediated way. A significant reduction in microvessel density and initial increase of tumor hypoxia on treatment with epothilone B (patupilone) alone was only detected in tumors derived from the MSA-sensitive A549 wild-type cells but not in the MSA-resistant cell line. These results indicate that the anti-angiogenic effect of patupilone *in vivo* is *indirectly* induced by inter-

ference at the level of the tumor cellular stress response.(62, 71) MSA interfere with the expression and transcriptional activity of the hypoxia-inducible factor 1α (HIF-1α) and subsequent reduction of the downstream pro-angiogenic HIF-transcriptome, including VEGF and other genes involved in angiogenesis, endothelial cell survival and hypoxic adaptation.(62, 63, 71) Vascular disruption as well as a decrease of the radioprotective effect of endothelial cells by VEGF and other survival factors will thereby enhance the radiation response, which is attributed to biological cooperation.(34, 60, 72)

Antimetastatic properties of MSAs (spatial cooperation). There is currently an ongoing discussion on the impact of ionizing radiation on tumor cell dissemination. Enhanced cell invasion and metastatic spread was observed in selected preclinical experiments in response to irradiation.(73-77) This effect is most probably due to IR-induced expression and secretion of matrix metalloproteinases (MMPs) required for cell invasion.(78-81) Interestingly the secretion of MMPs and related tissue inhibitors of metalloproteinases are also regulated by MSAs, as they are at least partially dependent on the dynamic functional MT system.(82, 83) At low doses, paclitaxel impairs the secretion of MMP-2 and MMP-9 by human melanoma and prostate cancer cells, thereby inhibiting cell invasion(84, 85) and this may be relevant mechanistically for the promising anti-metastatic effect of MSA. Furthermore, taxanes and epothilones can reduce migration of non-neoplastic smooth muscle and endothelial cells,(86-88) as well as neoplastic tumor cells (e.g. ovarian and colon carcinoma).(89, 90) The anti-metastatic properties of paclitaxel and epothilones were also demonstrated in tumor-bearing mice (for lung, prostate and breast tumors).(91-94)

Our own studies are currently investigating the combined treatment modality of epothilones and IR on MMP function. Interestingly, epothilones specifically counteract IR-induced MMP activity and IR-induced cell invasion of human fibrosarcoma and glioma cell lines.(95) This might represent an additional cause for the supra-additive tumor growth delay observed on combined treatment in preclinical *in vivo* experiments and is an interesting rational for a combined treat-

ment modality in the clinical setting.

The combined treatment modality of ionizing radiation with microtubule stabilizing agents fulfill the classic rationales of cell cycle specific enhancement, biological cooperation, temporal modulation and, in part, spatial cooperation. By causing cell cycle arrest in the most radiosensitive phase of the cell cycle, reducing the hypoxic fraction within the cellular population and thereby redistributing and reoxygenating the remaining tumor cells, and by interference with angiogenic signaling and endothelial cells, the interaction between microtubule stabilizing agents and ionizing radiation has a promising potential to increase the potency of this treatment combination. It is not only the additive effect of each of these mechanisms, which contribute to radiosensitization by MSAs, interestingly these mechanisms even complement each other on several levels thereby further enhancing the potency of this promising combined treatment modality.

Conflict of interest statement:

No potential conflicts of interest were disclosed.

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Figure legends:

Fig. 1.

A: Chemical structure: The MSA compounds are of complex structure and high chemical diversity. The complex structure of these compounds explains the difficulty for chemical synthesis.

B: Binding site: Microtubules are dynamic structures of α- and β-tubulin molecules arranged in tubular form. The microtubule-stabilizing agents of the taxane and epothilone groups bind along the interior surface of the microtubules to the same or an overlapping taxoid-binding site on β-tubulin. Thereby microtubular polymerization is enhanced and microtubular dynamics reduced.

C: Microtubules interact with various intracellular organelles: In the mitotic spindle, proper alignment and separation of the chromosomes during cellular division is provided by the normal microtubular function. Furthermore cellular structure and motion as well as vesicular transport take place by and along tubular structures.

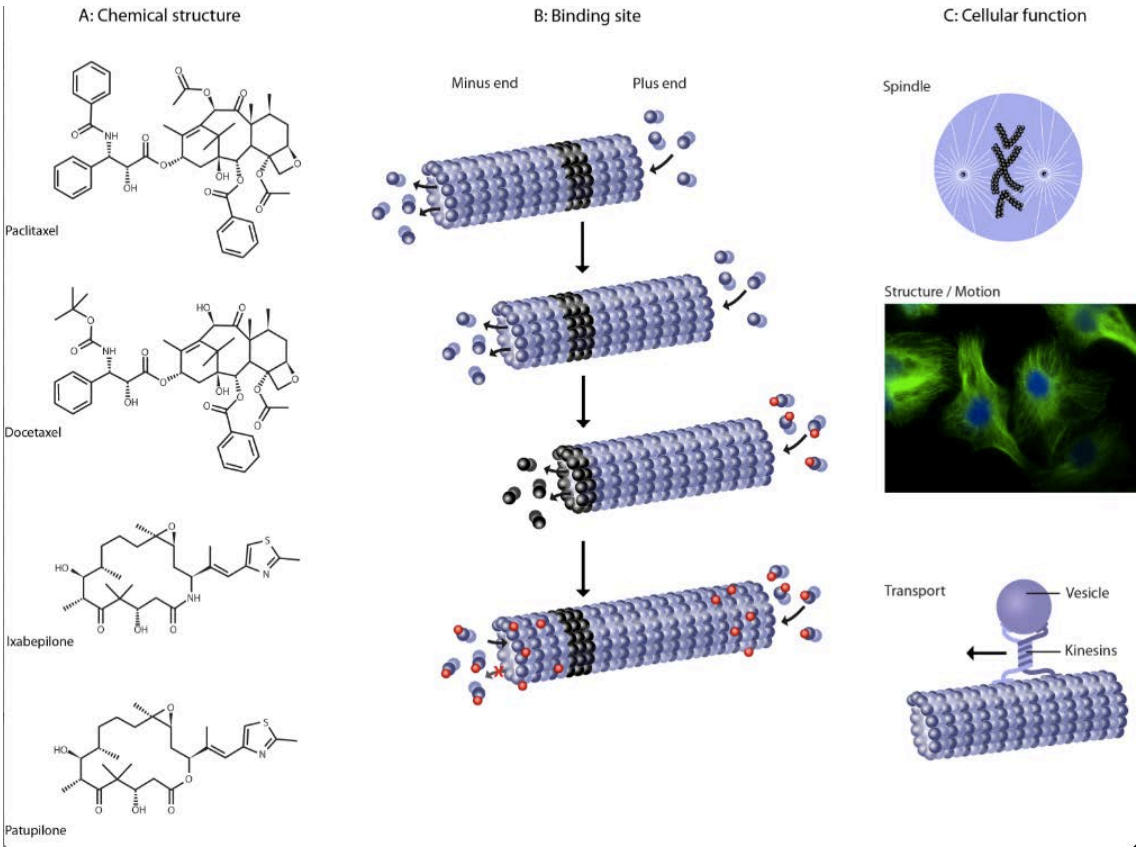


Fig. 2.

Multifaceted interaction of MSA and ionizing radiation on the tumor and endothelial cell level: 1. High doses of MSA and IR lead to reassortment of cells in the cell cycle, cell loss and subsequent tumor reoxygenation. Massive tumoral cell loss will directly decrease the metastatic potential. 2. MSAs counteract the pro-angiogenic IR-induced stress response. 3. MSAs counteract IR-induced secretion of survival and pro-metastatic factors thereby sensitizing the tumor to the combined treatment modality.

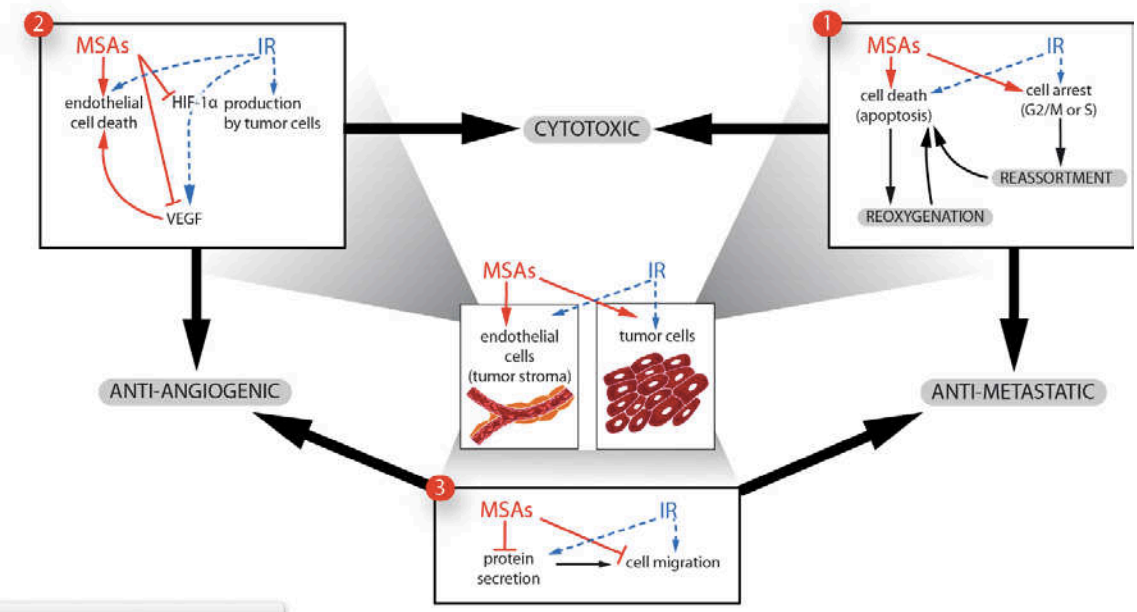


Fig. 3.

(Adapted from Hofstetter et al.,(55)). Effect of Patupilone and IR alone or in combination on the growth of SW480-derived xenografts in nude mice: Mice were treated with Patupilone (2 mg/kg, once) and IR (4 × 3 Gy, on 4 consecutive days), alone and in combination. Patupilone or the vehicle was administered 24 hours before the first fraction of IR. Combined treatment exerted a strong, supra-additive tumor growth control during treatment and the follow-up period. The human colon adenocarcinoma cell line SW480 is a p-glycoprotein (*MDR1*)-overexpressing tumor. Patupilone is a promising alternative in multidrug-resistant tumors for a combined treatment regimen using microtubule inhibitors and IR.

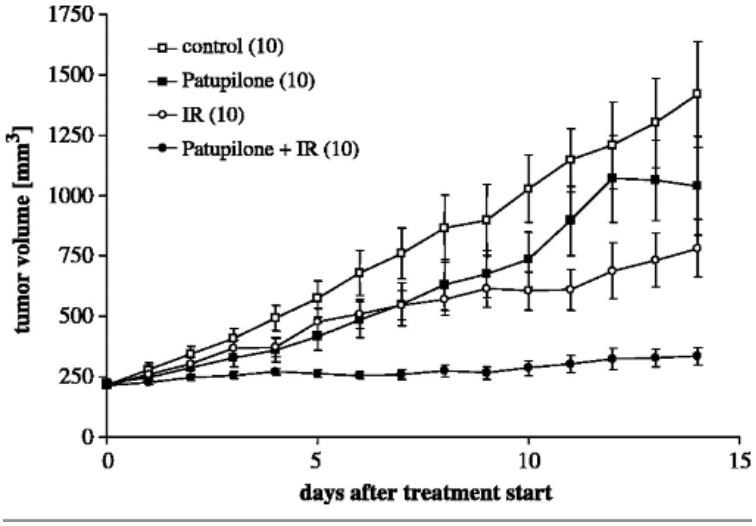
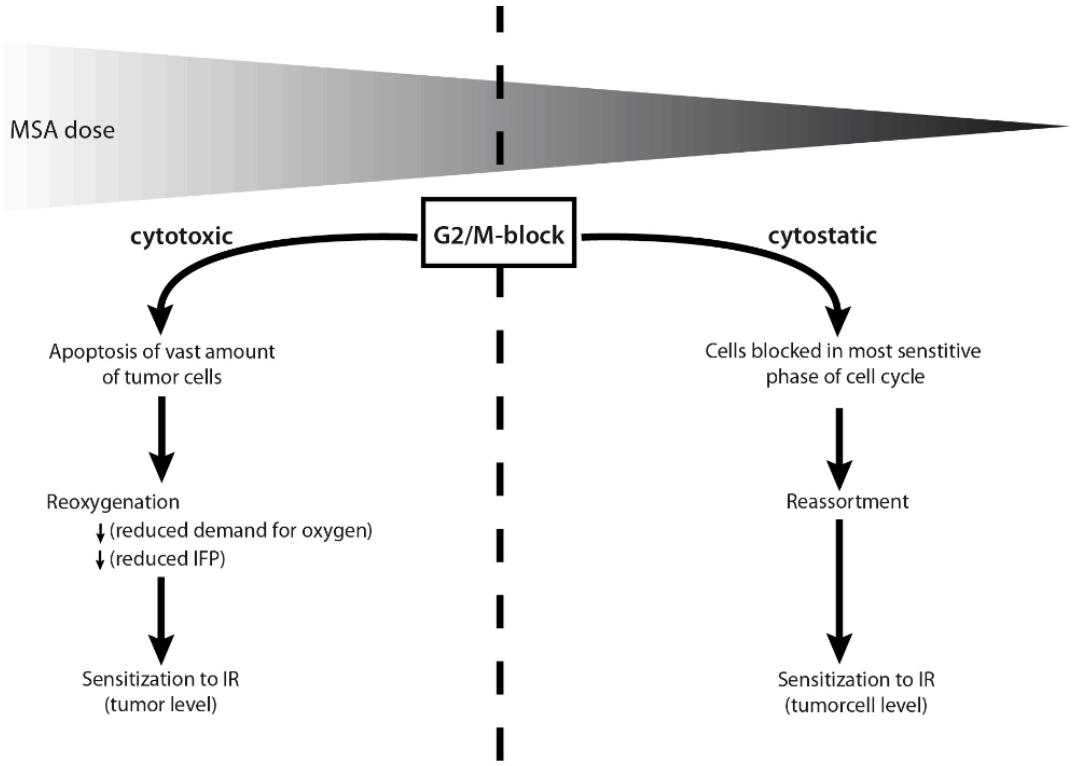


Fig. 4.

Dose dependent consequences of MSA via initial G2/M-phase block: cytostatic, low doses of MSA lead to cellular reassortment into the most radiosensitive phase of the cell cycle, while cytotoxic high doses of MSA radiosensitize the tumor by tumor reoxygenation.



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